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Regards
Bartgis McGlone.

V. THE ORIGIN OF THE LUNG OF AMPULLARIA

By WILLIAM KEITH BROOKS and BARTGIS McGLONE

Plates 1–7

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Plates 1–7

THE ORIGIN OF THE LUNG OF AMPULLARIA.

By W. K. Brooks and Bartgis McGlone.

INTRODUCTORY.

One of the authors was able to visit and partially explore the Everglades of Florida, in March, 1906, through the courtesy of the Director of the Marine Biological Laboratory of the Carnegie Institution of Washington, Dr. Alfred G. Mayer, in whose company the expedition was made. As we pushed our way through the tall reeds and grasses that cover the shallow water of the Everglades, we found great numbers of small eggs attached to the stems, above the surface of the water, but close to it (fig. 8, plate 1).

The eggs are arranged in vertical rows and inclosed in calcareous shells, resembling, in this respect, the eggs of the terrestrial pulmonate gasteropods. We also found, in the water, the prosobranchiate gasteropod, *Ampullaria,* in great abundance, and when some of the older eggs were opened, they were found to contain young ampullarias. The Paludinidæ, which are closely related to the Ampullaridæ, are aquatic, viviparous, and breathe by gills; and their structure seems to indicate that they are true prosobranchs, descended from, and closely related to, the marine prosobranchs. *Ampullaria* has gills, is partially aquatic, and seems to be a true prosobranch; but as it has a lung, and is able to breathe air and live out of the water, and as it also lays, in the air, eggs with calcareous shells like those of the terrestrial pulmonates, the question whether it, and the Paludinidæ, are primarily pulmonates with secondary resemblances to the prosobranchs, or primarily prosobranchs with secondary resemblances to the pulmonates, suggests itself. As the embryonic history of the organs of respiration may be expected to throw light upon this question, a quantity of eggs was collected and taken to the Marine Laboratory in the Tortugas. There the eggs were opened, the embryos removed, sketched and studied, and then hardened and preserved for more thorough examination.

Sections show that the lung is a member of the series of gill-filaments, and it must be regarded as a modified filament, or more than one. It is, therefore, a secondary acquisition which is not derived from the lung of the pulmonates.

Both lung and gill arise very early, and simultaneously, in the embryonic history. In a young embryo, soon after the mantle is formed, a ridge or thickening of the epithelium of its inner surface indicates the region where the gill-filaments, the osphradium, and the lung are to arise. The osphradium is developed from one end of this ridge, the gill-filaments from the other, and between the two the ridge becomes infolded into the substance of the mantle to give rise to the lung, which may be regarded as a modified and invaginated gill-filament. The similarity of the lung to that of the pulmonates is nothing more than a new illustration of resemblance between organs that have been acquired independently under like physiological conditions.

DESCRIPTIVE.

MATERIAL AND METHODS.

The investigation that is here described is based upon the study of the young stages in the development of an *Ampullaria* which occurs abundantly in the Everglades of Florida. It is identified as *Ampullaria depressa* Say by Professor Wm. H. Dall. The shell of the adult is shown of the natural size in plate 1, figures 1 and 2; and at two early stages in figures 3, 4, 5, and 6. Of these, 3 and 4 show the shell, enlarged, at the time when the young mollusk leaves the egg, while that of a young embryo is shown, much more enlarged, in 5 and 6. The eggs are laid, two or three inches above the level of the water, on the stems of the reeds and grasses, in vertical rows that are usually regular, as is shown in figure 8. Each is attached separately by a tenacious cement or glue. They are inclosed in white, chalky, calcareous shells. As the yolk is pink and, at first, fills the shell, while it is shrunken and partially replaced by air in the older eggs, these undergo a change of color during development, the younger ones being pink, while the older ones are white. It is clear that the attempt to distinguish species by separating ampullarias with pink eggs from those with white ones rests upon a misconception.

It is well known that *Ampullaria* has both gills and lung, and is adapted for both aquatic and aerial respiration. The lung is a large, elliptical, thin-walled pouch in the mantle, with an opening that is on the left side, above the left siphon, and immediately posterior to the osphradium. It is well shown in figures 5, 6, 7, and 8 of plate 1 of the Atlas of the Mollusca of the Voyage de l'Astrolabe.

The series of embryos that afforded the material for this research is complete, so far as the history of the respiratory organs is in question. In the younger ones the shell is a thin, transparent flat cap, and there are no traces of gills nor of lung; and the collection includes an abundance of embryos at each successive stage up to the time of hatching.

The young mollusks were kept alive in captivity for four weeks or more after hatching, but they did not undergo any observable change.

Those that were kept immersed drowned before the end of the second day, but those that were kept in damp air or were permitted to climb out of the water, remained in good health. The adults survive an immersion of a month or more without injury, but they leave the water occasionally when permitted to do so.

The embryos that have been studied were washed out of the broken shells by a stream of water, and they were fixed in picro-acetic acid or in formalin and preserved in 70 per cent alcohol. After the shells were removed by dilute nitric acid (8 to 15 drops in 100 c.cm. of alcohol) they were cut into sections in three planes, transverse, horizontal, and sagittal, a transverse section being one that is perpendicular to the long or principal axis of the extended embryo; horizontal, one that is parallel to the principal axis and at right angles to the median plane of morphological symmetry; sagittal, parallel to the principal axis and to the median plane of symmetry.

Haidenhain's hematoxylin was used for staining on the slide, with eosin as counterstain. Excellent results were obtained by shortening time. For instance, the alum bath was used for one or two hours, and the hematoxylin for only half an hour. Embryos that were to be mounted whole were stained in acid borax carmine and cleared with clove oil.

THE GENERAL ANATOMY OF THE EMBRYO.

In order to make the account of the development of the organs of respiration intelligible, it should be preceded by an outline sketch of the general anatomy of the embryos.

The anatomy of the embryo, at the time when the first traces of the organs of respiration are found, will be understood from plate 1, figure 7, and plate 3, figure 11. Plate 1, figure 7, is a surface view of a young embryo, and plate 3, figure 11, a reconstruction of a series of oblique sagittal sections of a slightly older one. Both are of the same length, 1.2 mm.

The anterior region consists of a great head-vesicle, *hv,* and the foot, *f.* In the ventral anterior region of the vesicle is a lipped opening, the mouth, *m.* Above this, and encircling the vesicle, is a ciliated ridge, the velum, *v.* The foot, *f,* is longer than broad, and its lower surface is flattened. On its posterior border are a few large, clear cells, apparently calcareous, three of which are shown in plate 1, figure 7. In young embryos they are large and numerous, but they disappear as growth progresses, and none are to be found at the stage shown in plate 3, fig. 11. On the dorsal surface of the posterior region of the foot the ectoderm becomes thickened and secretes a calcareous operculum, *o.* The posterior region of the body, with the mantle, mantle-chamber, and shell, is shown in front view in plate 1, figure 7, and in section in plate 3, figure 11. It resembles a bowl in shape, with the inner concave surface facing to the right. The convex left-hand surface is composed of the thickened ectoderm that secretes the shell, *sh.*

It is surrounded by a folded ridge, *mf,* the margin of the shell-gland. In plate 3, figure 11, the shell covers the whole of the posterior end of the body, and the raised edges of the shell-gland are pushed far apart. The growth of the shell brings about changes in the position of the mantle-chamber, in that of the mantle itself, and in that of other organs, as will be noted later.

From the mouth the œsophagus, *oe,* runs upward, within the head-vesicle, to the dorsal anterior region, where it opens into a huge chamber, the primitive stomach, *pr. st.,* which fills most of the head-vesicle. The sac of the radula (*r,* fig. 11) arises from the posterior (ventral) border of the œsophagus near the mouth, and extends under the ventral surface of the primitive stomach. A pair of salivary glands that are not shown in the figure arises from the œsophagus, near the mouth, as a pair of pouches or outgrowths. The primitive stomach narrows posteriorly and gives rise to the intestine, which, arising on the left, bends to the right, and opens into the cavity of the mantle, through the anus, *a,* which is at first median and ventral, but moves upward on to the right side of the mantle as development advances. Near the anus there is, in young embryos, a rosette-shaped cluster of large transparent cells like those that have been mentioned on the foot. They persist until about the time of hatching. The buccal and cerebral ganglia arise as compact groups of spindle-cells below and above the radula, while a similar group of cells arises in the posterior part of the foot and becomes the pedal ganglia.

The heart is shown in plate 3, figure 11, near the bottom of the figure; although the opening through which the auricle, *au.,* communicates with the ventricle, *ven,* does not lie in the plane of this section. The heart is inclosed in a spacious pericardium, *per,* with a thin wall of spindle-shaped mesoderm-cells. The aortic sinus, which arises as a split in the mesoderm, is shown at *a. s.* in figure 11, which cuts it in the region of the foot and again close to the pericardium. The right kidney, which is functional in the adult, is shown in figure 11 at *k.* It, as well as the abortive left kidney, arises as an evagination from the pericardium, while its duct, which is far to the right of the plane of the section, arises as an invagination of the inner surface of the mantle where this becomes continuous, on the extreme right, with the body-wall.

THE DEVELOPMENT OF THE ORGANS OF RESPIRATION.

In the adult these are on or under the inner surface of the mantle, and consist of the lung, the right gill, and the osphradium; and they stand in intimate anatomical relation to the heart and the renal organ. The lung is a large, elliptical, thin-walled pouch which opens into the chamber of the mantle through an aperture that is protected by valves. The opening is on the left side of the mantle above the left siphon and posterior to the osphradium—a small, oblong, laminated organ, about 3 mm. long. The gill extends along the right boundary of the lung, from which it is incompletely separated by a fold or ridge on the body (plate 5, fig. 15; also plate 57, fig. 6, Atlas of the Mollusca of the Voyage de l'Astrolabe). Anteriorly the gill diverges from the lung and ends, together with it, on the extreme left, dorsal to the heart, into the auricle, of which the veins of both organs enter through a common trunk.

The earliest stage in the development of the organs of respiration is shown, in surface view, in plate 1, figure 7, at *resp. rud.;* and, in section, in plate 2, figure 9. It makes its appearance as two parallel ridges, *g. r* and *o. r*, separated by a furrow, *l. r.* As the section shows, the ridges arise as thickenings of the epithelium of the mantle. One of them, *g. r,* is the rudiment of the gill, and the other (*o. r*) the rudiment of the osphradium; while the furrow, *l. r,* is the first trace of the lung, which is ciliated. The substance of the mantle, between the inner layer of epithelium which gives rise to the organs of respiration and the outer layer which forms the shell-gland, *sg,* is loosely filled with mesoderm, which is derived from the ectodermal epithelium by migration. At the stage that is shown in the figure the mesoderm is so arranged as to leave an unoccupied space, the pulmonary sinus, *p. s.*

A section of a stage that is somewhat older is shown in figure 10. Comparison with figure 9 shows that the gill-filament, *g. r.,* grows through the thickening and multiplication of the epithelial cells together with the multiplication of the underlying mesoderm. In the region of the lung, *l. r.,* the thickening and multiplication of the epithelium are of such character that they lead to an infolding instead of an outpushing, as is illustrated by the figure. An older stage, with traces of three gill-filaments, is shown in plate 3, figure 11. This is a reconstruction from a series of oblique sagittal sections of an embryo about 1.2 mm. long, with a shell that is nearly hemispherical, *sh.*

The lung, *l. r.,* is now deeply infolded and there are three gill-folds, *r. g. f.* The pulmonary sinus, *p. s,* is now a well-defined chamber under the respiratory organs, and it now opens into the auricle, *au,* by an aperture that is not shown in the figure. The gill-filaments are filled with mesoderm in which there is as yet no indications of a cavity. The mantle, mantle-chamber, and respiratory organs, at this stage, are shown, more enlarged, in plate 4, figure 12. The gill-filaments are filled with a compact mass of mesoderm, in the plane of the section, although this is looser to the right of the section and the interspaces communicate with the pulmonary sinus, *p. s.* The lung, *l. r.,* bends to the left as far as the osphradium, which is now nearly round.

The chamber of the mantle of an older ambryo, 1.4 mm. long, is shown in plate 5, figure 14, in order to illustrate the relation of the respiratory organs to other structures; while these organs are shown, more enlarged, in plate 4, figure 13, to illustrate the details. They are from an embryo with a shell that nearly half covers it, with knob-like tentacles with eyes, and other approximations to the structure of the adult. The gill runs obliquely from left to right, and its filaments are visible through the shell in an entire embryo.

Figure 14 is a transverse section through the posterior half of this embryo. On the right, near the lower point of the mantle-chamber (near the top of the figure), is the rectum, *rec;* a cylindrical tube lined with columnar epithelium. Above the rectum (to the right of it in the figure) is a well-defined group of spindle-shaped mesoderm cells, the kidney, *k.* On the right (bottom of the figure) is the thin-walled pericardium, *per.* Above (to the right of) this is the pulmonary sinus, *p. s.* The gill-filaments, *g. f,* now project freely into the chamber of the mantle, *m. c,* the newer ones arising on the left (below in the figure) between the older filaments and the lung, *l. g,* which is a deep groove running forwards (downwards in the figure). The gills and lung are shown, more enlarged, in plate 4, figure 13. The gill-filaments are outfoldings of the columnar epithelium of the mantle, and they are loosely filled with a tissue of spindle-shaped mesoderm cells, between which are spaces that communicate with the pulmonary sinus, *p. s.* The lung-groove is lined with elongated ectoderm cells covered, on its internal surface, by a cap of mesoderm cells which arise from the ectoderm, as the illustration shows.

The chamber of the mantle, with the lung, *l.,* the gill, *g. f,* and the adjacent organs, are shown, at four successive stages of advanced development, in plate 6, figure 17; plate 6, figure 16; plate 7, figure 18, and plate 5, figure 15; the last figure showing the respiratory organs at the time of hatching. In this series, figure 16 is from an embryo 1.8 mm. long. The shell is shown, at this stage, in plate 1, figures 5 and 6, with one complete turn in the spiral.

The section shown in figure 17 is one that, if continued, would pass through the stomach, the liver, the operculum, and the part of the foot that is ventral to the operculum. The part of the section that is figured shows, besides the respiratory organs, the rectum, *rec;* the kidney, *k;* the osphradium, *osph,* and part of the pericardium, *per.* The lung, *l.,* is a deep pit, with an opening into the chamber of the mantle and is constricted by a projecting fold, *r,* of the respiratory region. While figure 16 is from an older embryo, it shows the anatomical relations that would be shown in another section of the stage shown in figure 17, and the two figures may be regarded as showing the lung, *l.,* in two sections of a single specimen. In figure 16 the cavity of the lung, *l.,* occupies nearly the whole thickness of the mantle, so that its lining of epithelium is separated from the epithelium of the shell-gland only by a thin layer of mesoderm. In this figure the intestine, *int,* is shown as a fold in the wall of the stomach. Figure 18 is from an embryo about 2.1 mm. long, with a shell like that which is shown in plate 1, figures 3 and 4. The lung is deeply infolded and the section cuts nine gill-filaments, *g. f.*

Plate 5, figure 15, is from a young *Ampullaria*, at the time of hatching, with its organs of respiration in their definitive adult condition. The lung, *l*, is now a spacious chamber occupying most of the thickness of the mantle and opening into its chamber by a small, closable aperture.

At about this time the gill and the lung become separated from each other by a valvular fold (*part*) which grows out from the body into the chamber of the mantle.

Summary.—The gills, the lung, and the osphradium of *Ampullaria* arise simultaneously, or nearly so, in the embryo; and they are developed out of a ridge or thickening of the epithelium of the mantle. They must, therefore, be regarded as a series of homologous organs specialized among themselves in different directions, and not as independent acquisitions. There is no reason to think that there is any ancestral connection or relationship between the lung of *Ampullaria* and that of the pulmonates, although the embryonic history of the lung of *Ampullaria* shows that the origin of the lung of the pulmonates through the modification of a gill, or part of a gill, is not impossible. The lung of *Ampullaria* becomes functional before the gill does; for the newly hatched young die very quickly if they are prevented from leaving the water, while the adults survive a long immersion.

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DESCRIPTION OF PLATES.

Letters of Reference.

an.	Anus.
a. s.	Aortic sinus.
au.	Auricle.
f.	Foot.
g. r.	Rudimentary gill.
r. g. f.	Rudimentary gill filament.
g. f.	Gill filament.
r.	Fold that forms part of floor of lung.
h. v.	Head vesicle.
k.	Kidney.
l. r.	Rudimentary lung.
l. g.	Lung groove.
l.	Lung.
m.	Mouth.
m. c.	Cavity of mantle.
m. f.	Edge of shell gland.
n.	Nerve tissue.
oe.	Œsophagus.
o.	Operculum.
o. r.	Rudimentary osphradium.
osph.	Osphradium.
part.	Partition of cavity of mantle.
per.	Pericardium.
p. s.	Pulmonary sinus.
p. v.	Pulmonary vein.
r. s.	Radula sac.
rec.	Rectum.
resp. rud.	Rudiment of organs of respiration.
sh.	Shell.
sh. g.	Shell gland.
p. st.	Primitive stomach.
st.	Stomach.
v.	Velum.
ven.	Ventricle.

EXPLANATION OF PLATES.

PLATES 1 TO 7.

All of the figures are of *Ampullaria depressa* Say.

PLATE 1.

FIGS. 1 and 2. Adult, natural size.
FIGS. 3 and 4. Enlarged view of shell at time of hatching.
FIGS. 5 and 6. Enlarged view of shell of unhatched embryo 1.8 mm. long.
FIG. 7. Surface view of an unhatched embryo 1.2 mm. long.
FIG. 8. Eggs attached to grass.

PLATE 2.

FIGS. 9 and 10. Sections of the organs of respiration in their earliest stages. Figure 10 shows a stage somewhat older than that illustrated in figure 9.

PLATE 3.

FIG. 11. Reconstruction from a series of oblique sagittal sections of an embryo in the stage shown in figure 7, plate 1.

PLATE 4.

FIG. 12. Sagittal section of mantle-chamber and respiratory organs in an embryo 1.2 mm. long.
FIG. 13. Transverse section of the mantle-chamber of an embryo 1.4 mm. long.

PLATE 5.

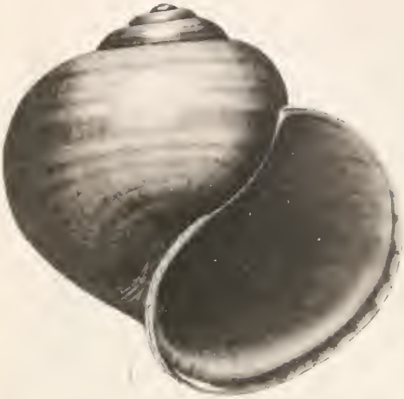
FIG. 14. Transverse section of the mantle chamber of an embryo 1.4 mm. long.
FIG. 15. Section of the respiratory organs at the time of hatching.

PLATE 6.

FIGS. 16 and 17. Section of mantle-chamber showing lung, gill, etc. Figure 16 shows a stage wherein the embryo is 1.8 mm. long and is older than that shown in figure 17.

PLATE 7.

FIG. 18. Section of an unhatched embryo 2.1 mm. long.



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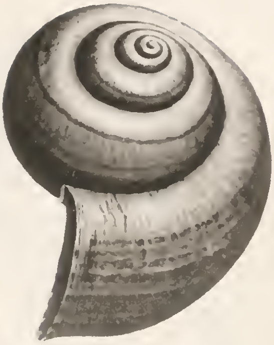

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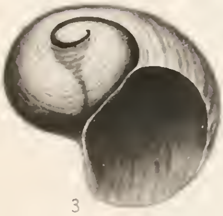

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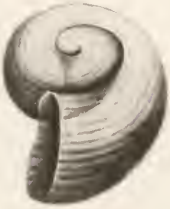

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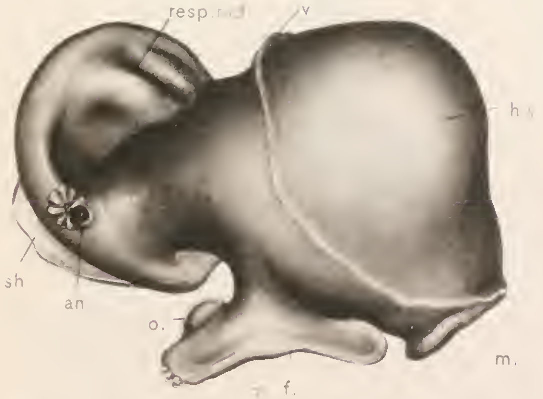

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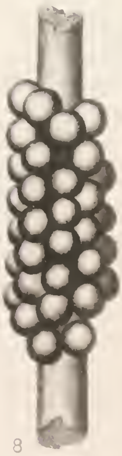

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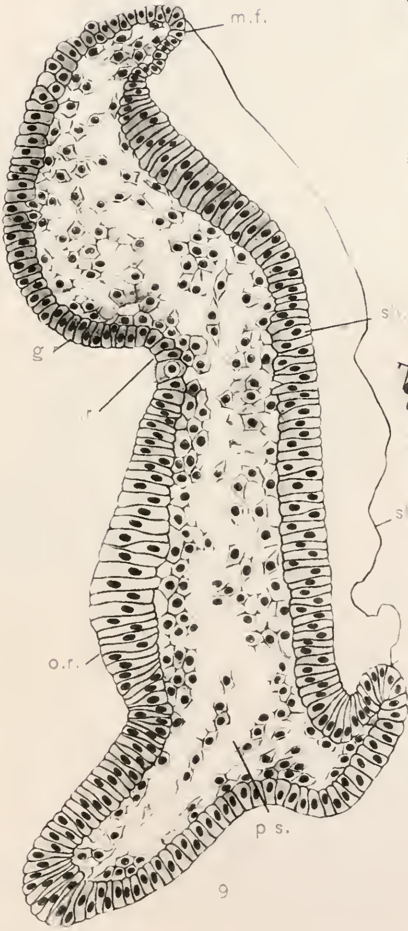

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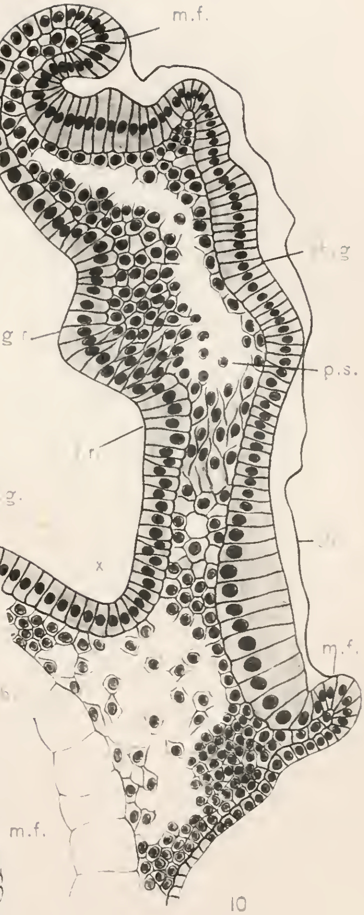

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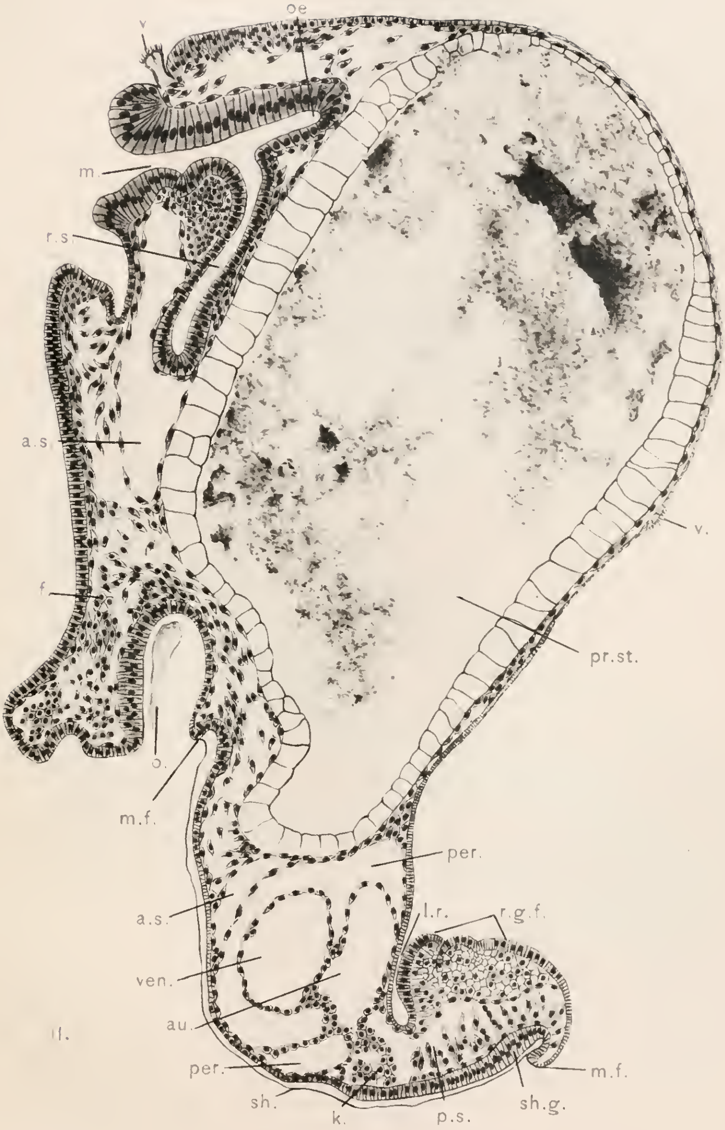

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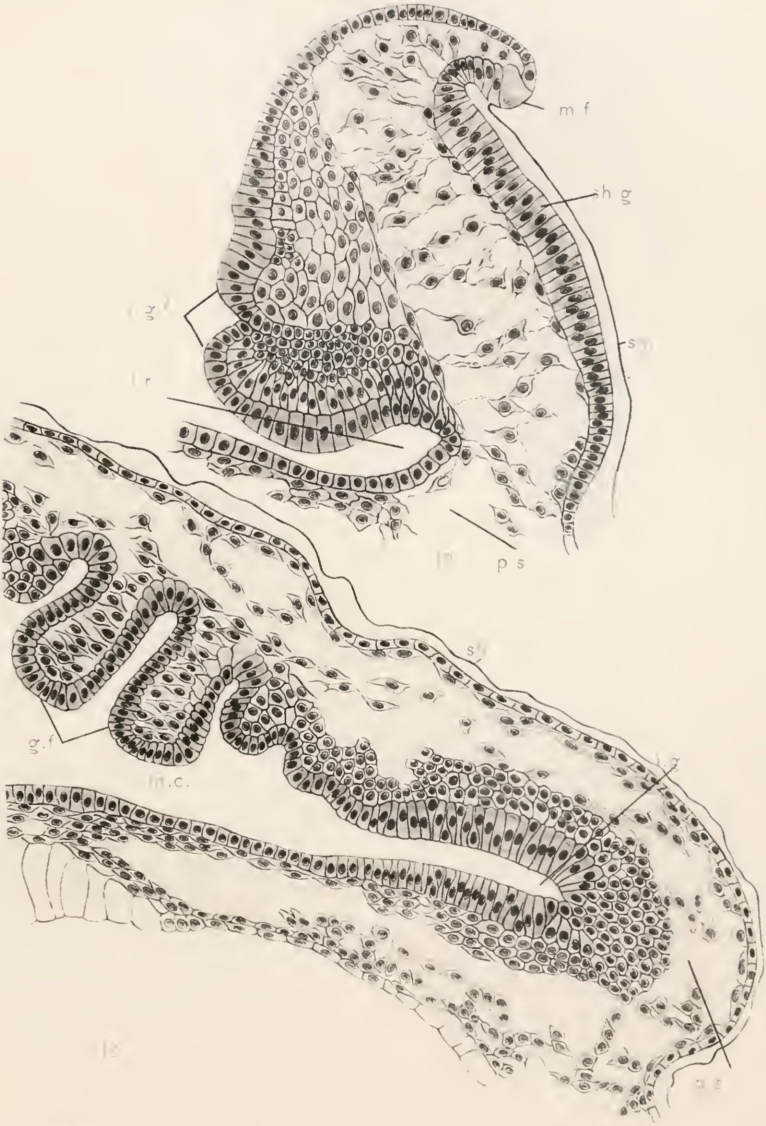

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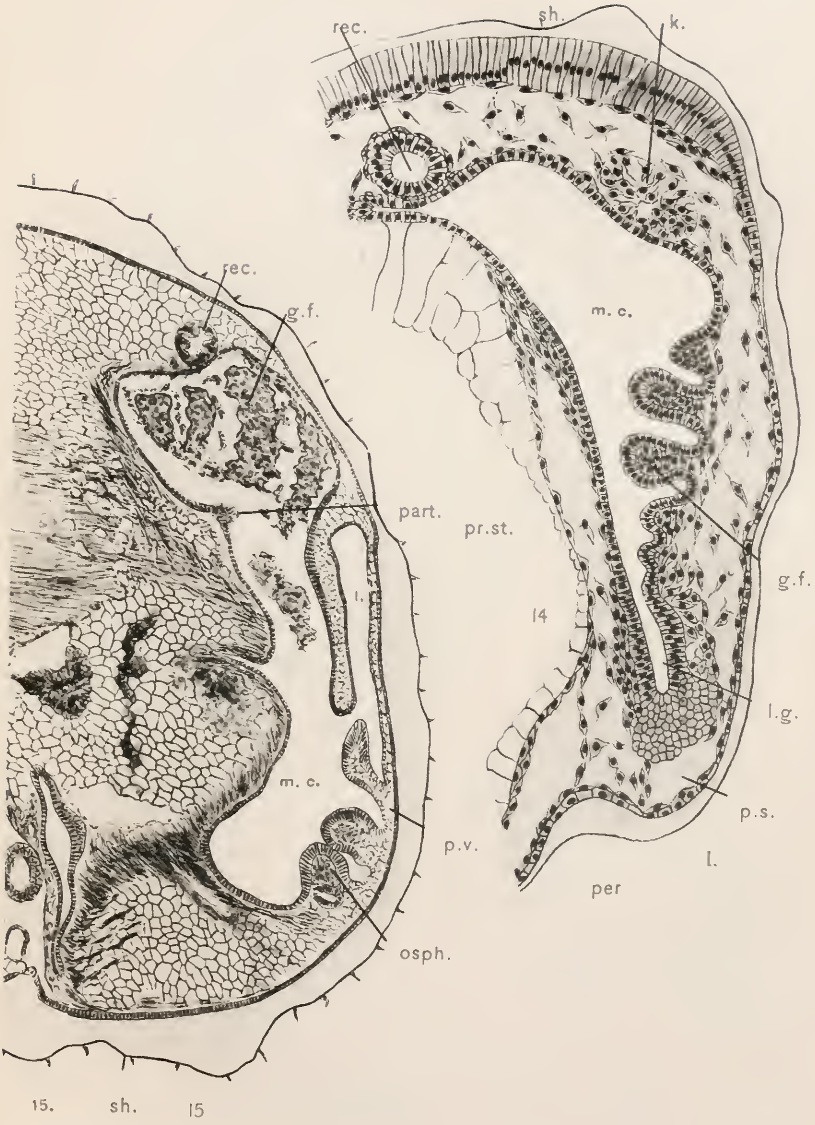

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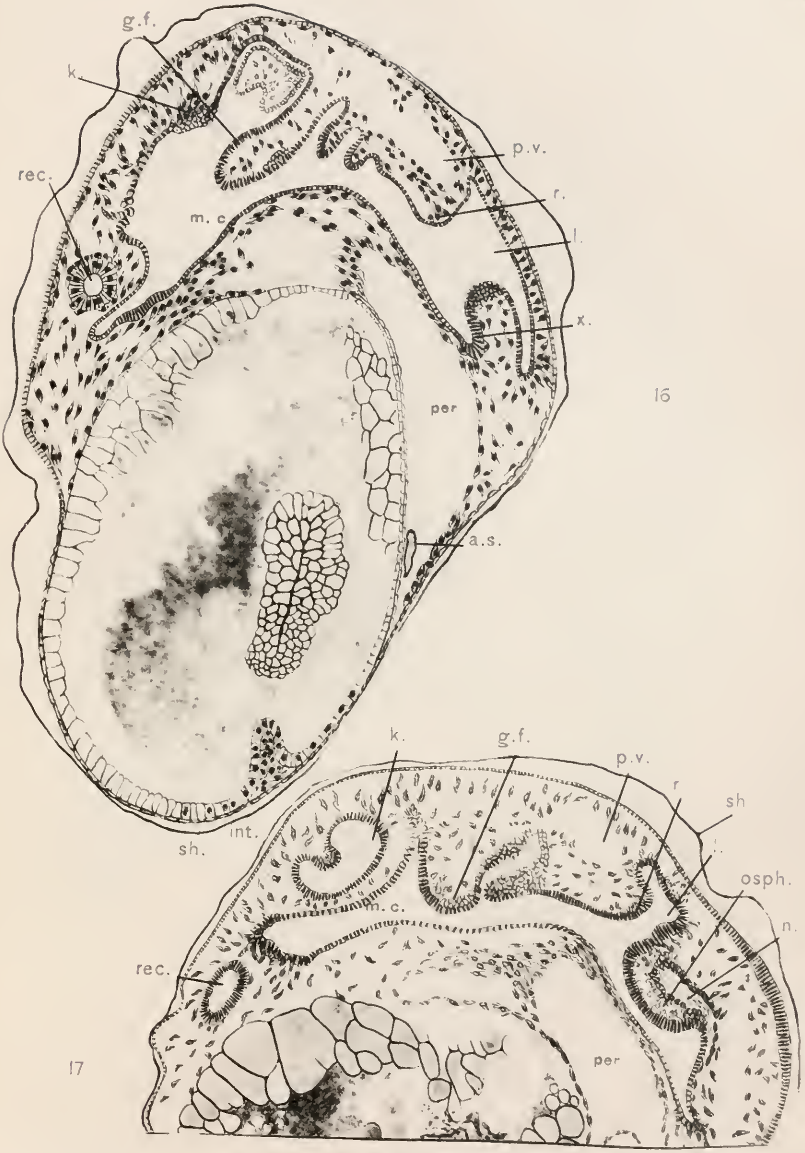
BROOKS AND MAC GLONE. PLATE 5



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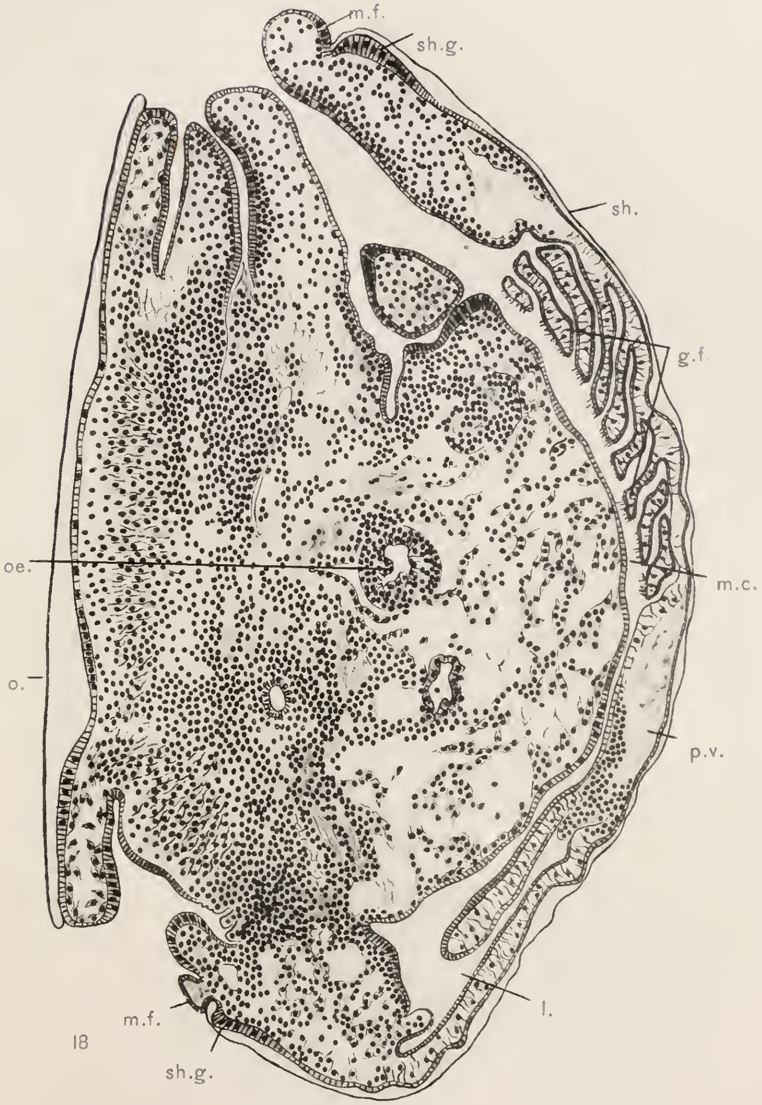

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